2000

Role of Vascular Endothelial Growth Factor in Placental Vascularization

Kimberly A. Vonnahme
Iowa State University

Matthew E. Wilson
Iowa State University

Stephen P. Ford
Iowa State University

Extension Number: ASL R1666

Role of Vascular Endothelial Growth Factor in Placental Vascularization

Abstract
The ratio of a fetus's weight to that of its placenta has been used in our laboratory as an estimate of placental efficiency, which defines the number of grams of placenta required to support a gram of fetus. Because the pig placenta is noninvasive, nutrients from the mother must diffuse from uterine blood vessels to placental blood vessels at the placental-endometrial interface. A pig placenta can respond to increasing fetal nutrient demands by either increasing in size, and thus surface area in contact with the endometrium, or by increasing the number of blood vessels per unit area at the fetal-maternal interface. Previous studies from our laboratory have shown Meishan and Yorkshire conceptuses gestated in a Meishan uterus had markedly smaller placentae than Meishan or Yorkshire conceptuses gestated in a Yorkshire uterus whereas fetal weights across the two uterine environments were much more similar. These data suggested that conceptuses in a Meishan uterine environment have a greater vascular density at the placentalendometrial interface. Greater densities of blood vessels can be achieved by either vasodilation (increasing the diameter of existing blood vessels) or angiogenesis (growth of new vessels from preexisting ones). Hypoxia, or inadequate oxygen transport, has been shown to increase angiogenic factors, such as vascular endothelial growth factor (VEGF), which stimulate blood vessel development. It is possible that VEGF may be important in stimulating increased blood vessel density in the pig placenta, as it has been to shown to do in the ovine placenta. Our objective was to determine if VEGF mRNA expression was associated with placental and/or endometrial vascular density, placental efficiency, and litter size during late gestation. We observed a positive association of both placental vascular density ($r=0.37$, $p<0.05$) and VEGF mRNA levels ($r=0.35$; $P<.05$) with placental efficiency . There was also a positive correlation between VEGF mRNA levels and the number of conceptuses in a litter ($r=0.42$; $P<.05$) on day 70, 90, and 110 of gestation. Although Yorkshire uteri exhibited greater endometrial vascular density than Meishan uteri on day 70 of gestation, placentae of conceptuses gestated in a Meishan uterus had greater amounts of VEGF mRNA than placentae of conceptuses gestated in a Yorkshire uterus. The greater amounts of placental VEGF mRNA of conceptuses gestated in a Meishan uterus on day 70 may have resulted in the increased placental vascular density observed on day 90. We conclude that the increased litter size of the Meishan female may stem from her having smaller, more vascular placentae than placentae of conceptuses gestated in the uterus of a Yorkshire female, to allow for efficient nutrient delivery to the fetus. The increased placental vascular density of a conceptus gestated in the uterus of a Meishan female may result from increased placental VEGF mRNA production induced from an endometrial induced hypoxia on day 70 of gestation.

Keywords
ASL R1666

Disciplines
Agriculture | Animal Sciences

Role of Vascular Endothelial Growth Factor in Placental Vascularization

Kimberly A Vonnahme, graduate assistant,
Matthew E. Wilson, graduate assistant,
and Stephen P. Ford, professor,
Department of Animal Science

ASL-R1666

Summary and Implications

The ratio of a fetus's weight to that of its placenta has been used in our laboratory as an estimate of placental efficiency, which defines the number of grams of placenta required to support a gram of fetus. Because the pig placenta is noninvasive, nutrients from the mother must diffuse from uterine blood vessels to placental blood vessels at the placental-endometrial interface. A pig placenta can respond to increasing fetal nutrient demands by either increasing in size, and thus surface area in contact with the endometrium, or by increasing the number of blood vessels per unit area at the fetal-maternal interface. Previous studies from our laboratory have shown Meishan and Yorkshire conceptuses gestated in a Meishan uterus had markedly smaller placentae than Meishan or Yorkshire conceptuses gestated in a Yorkshire uterus whereas fetal weights across the two uterine environments were much more similar. These data suggested that conceptuses in a Meishan uterine environment have a greater vascular density at the placental-endometrial interface. Greater densities of blood vessels can be achieved by either vasodilation (increasing the diameter of existing blood vessels) or angiogenesis (growth of new vessels from preexisting ones). Hypoxia, or inadequate oxygen transport, has been shown to increase angiogenic factors, such as vascular endothelial growth factor (VEGF), which stimulate blood vessel development. It is possible that VEGF may be important in stimulating increased blood vessel density in the pig placenta, as it has been to shown to do in the ovine placenta. Our objective was to determine if VEGF mRNA expression was associated with placental and/or endometrial vascular density, placental efficiency, and litter size during late gestation. We observed a positive association of both placental vascular density ($r=0.37$, $p<0.05$) and VEGF mRNA levels ($r=0.35$; $P<.05$) with placental efficiency . There was also a positive correlation between VEGF mRNA levels and the number of conceptuses in a litter ($r=0.42$; $P<.05$) on day 70, 90, and 110 of gestation. Although Yorkshire uteri exhibited greater endometrial vascular density than Meishan uteri on day 70 of gestation, placentae of conceptuses gestated in a Meishan uterus had greater amounts of VEGF mRNA than placentae of conceptuses gestated in a Yorkshire uterus. The greater amounts of placental VEGF mRNA of conceptuses gestated in a Meishan uterus on day 70 may have resulted in the increased placental vascular density observed on day 90. We conclude that the increased litter size of the Meishan female may stem from her having smaller, more vascular placentae than placentae of conceptuses gestated in the uterus of a Yorkshire female, to allow for efficient nutrient delivery to the fetus. The increased placental vascular density of a conceptus gestated in the uterus of a Meishan female may result from increased placental VEGF mRNA production induced from an endometrial induced hypoxia on day 70 of gestation.

Introduction

Chinese Meishan pigs farrow three to four more piglets per litter compared with U.S. breeds despite having a similar ovulation rate and uterine size. This increase in litter size results both from an increased conceptus survival to day 30 and their continued survival to term. To investigate fetal breed differences on conceptus growth and survival in different uterine environments, reciprocal embryo transfer studies between Meishan and Yorkshire females were conducted. We found that although both fetal and placental weights were reduced for conceptuses gestated in Meishan uteri compared with conceptuses gestated in Yorkshire uteri, placental weights differed far more than fetal weights.

The placenta can respond to increasing fetal nutrient demands by increasing its surface area of association with the uterine wall or by remaining the same size and increasing the density of blood vessels at the placental-endometrial interface. To achieve increases in densities of blood vessels, vasodilation (existing blood vessels increasing in diameter) or angiogenesis (growth of new blood vessels from preexisting vessels) must occur. Our laboratory has defined placental efficiency as the ratio of fetal to placental weight, which estimates the of the number of grams of placenta required to support a gram of fetus. As previously reported from our laboratory, changes in the placental efficiency of the pig conceptus result from changes in placental weight, not fetal weight.

Increasing nutrient demands of the growing fetus may result in conceptus differences in placental vascularity and placental efficiency due to differences in an angiogenic response. Vascular endothelial growth factor (VEGF), a potent angiogenic factor, has been found in the ovine placenta during late gestation at a time of rapid vessel growth. Hypoxia, or inadequate oxygen transport, resulting from increased fetal nutrient demands has been shown to increase VEGF gene expression in the ovine placenta.

It was our objective to determine if levels of placental VEGF gene expression were associated with placental and/or adjacent endometrial vascular density, placental efficiency and litter size when Meishan or Yorkshire conceptuses were gestated by either Meishan or Yorkshire females to days 70, 90, and 110 of gestation.

Materials and Methods

Meishan and Yorkshire gilts were checked twice daily for estrus. Donor gilts (n=12) were hand-mated to boars of their own breed at the onset of estrus (day = 0) and 12 hours later. Embryos were transferred into gilts that were synchronous with donor gilts. Meishan recipients (n=6) received embryos only from Yorkshire donors and Yorkshire recipients (n=6) received embryos only from Meishan donors on day 2.5 of the estrous cycle. Additional groups of Meishan (n=6) and Yorkshire (n=6) females were hand mated to boars of their own breed and allowed to gestate their own conceptuses.

Two females that represented each of the maternal breed _ conceptus breed groups (Meishan females carrying either Meishan or Yorkshire conceptuses and Yorkshire females carrying either Meishan or Yorkshire conceptuses) were slaughtered on day 70, 90 or 110 of gestation. At slaughter, litter size, fetal weight, and placental weight were determined. Placental tissue was immediately frozen on dry ice and stored at -80☜C until RNA extraction. A section of the maternal- fetal interface located ~4 cm from the umbilical cord was fixed in formaldehyde and embedded in paraffin. Blocks of tissue were then cut into five μm sections via a microtome, and sections stained to allow for calculation of vascular density. Percentage of placental vascular density and endometrial vascular density were determined by image analysis. Briefly, a single image of each of three separate microscopic fields located along the chorioallantoic-endometrial contract area was viewed via a video camera mounted on a microscope. The area occupied by placental and endometrial tissue was measured. The area occupied by all blood vessels in the placental tissue was measured whereas only endometrial blood vessels that occupied the area between the endometrial glands and the placental endometrial interface were measured. Percentage of placental vascular density was determined by dividing the area of the placental blood vessels by the total placental area. Percentage of endometrial vascular density was determined by dividing the area of the measured endometrial blood vessels by the total endometrial area.

Measuring the amount of VEGF gene expression, as estimated by VEGF mRNA levels, gives an indication of how much VEGF protein will be made that could result in increasing the density of blood vessels. Levels of VEGF gene expression were quantified by ribonuclease protection assays. Each sample was corrected to a constant level with the housekeeping gene, pyruvate dehydrogenase. At least two aliquots of a placental RNA pool were run on each gel to correct for gel to gel variation.

Results

As observed in previous studies from our laboratory, regardless of fetal breed, conceptus growth was inhibited in the Meishan uterine environment compared with conceptuses gestated in a Yorkshire uterine environment. Although fetuses were 24% lighter when gestated in Meishan uteri than Yorkshire uteri to day 70, 90, and 110, placentae were 42% lighter in Meishan uteri compared with Yorkshire uteri.

Placental efficiency was positively correlated (r= 0.37; P<.05) with placental vascular density, thus the smaller the placenta, the more vascular the placenta. Additionally, there was a positive correlation (r = 0.35; P<.05) between placental efficiency and VEGF mRNA levels.

There were no overall differences in placental VEGF mRNA levels between Meishan and Yorkshire conceptuses. However, levels of placental VEGF mRNA were greater when conceptuses of either breed were gestated in a Meishan compared with a Yorkshire uterus.

As can be seen in Figure 1, significantly greater endometrial vascular density is seen in Yorkshire uteri on day 70 compared with Meishan uteri. Both breeds reach similar degrees of endometrial vascularity by day 90, which

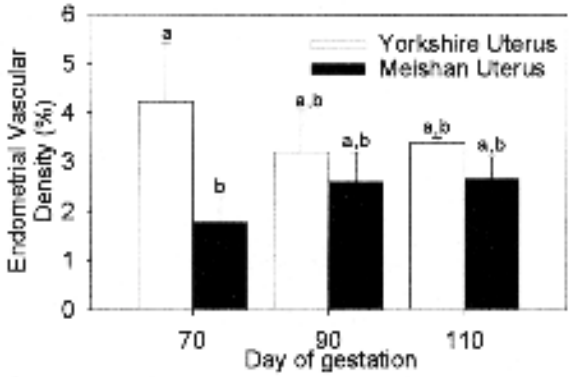

Figure 1. Endometrial vascular density adjacent to placentae of conceptuses gestated in Yorkshire and Meishan uteri. Means ± SE with different letters differ (P<.05).

continues to day 110. Figure 2 demonstrates that conceptuses gestated in Meishan uteri contain markedly greater levels of placental VEGF mRNA on day 70 than placentae of conceptuses gestated in Yorkshire uteri. Placentae of conceptuses in both Meishan and Yorkshire uteri increase their level of VEGF mRNA from day 70 to day 90, which remain constant to day 110.

Although placental vascular density remained unchanged from day 70 through day 110 when conceptuses are gestated in a Yorkshire uterus, placentae of conceptuses gestated in a Meishan uterus increased their vascular density ~4 fold from day 70 to 90, then remained unchanged through day 110.

By averaging placental VEGF mRNA levels in a litter, it was observed that the level of VEGF mRNA in placental tissue is positively associated (r=0.42; P<.05) with the number of conceptuses in that litter.

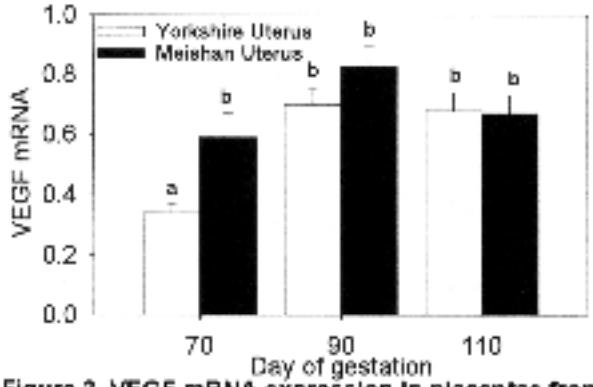

Figure 2. VEGF mRNA expression in placentae from conceptuses gestated in either a Yorkshire or Meishan uterus on day 70, 90, and 110 of gestation. Means ± SE with different letters differ (P<.05).

Discussion

We have demonstrated that pig placentae express VEGF mRNA during late gestation. Furthermore, VEGF mRNA in placental tissue is positively associated with placental efficiency and litter size. Placental efficiency, an index of placental size, is associated with placental vascular density.

As previously mentioned, although fetuses were lighter (24%) when gestated in a Meishan uterus compared with fetuses gestated in a Yorkshire uterus, placentae of conceptuses gestated in a Meishan uterus were markedly lighter (42%) than placentae gestated in a Yorkshire uterus. Because greater placental efficiency is a result of a reduced placental size rather than a change in fetal weight, it appears logical that placental vascular density must increase in the smaller placenta to meet the increasing nutrient demands of the fetus.

On day 70 of gestation, there is a markedly lower in endometrial vascular density in Meishan versus Yorkshire females. The lower endometrial vascular density in the Meishan uterus may result in a hypoxic condition that increases placental production of VEGF mRNA. This increased level of placental VEGF mRNA on day 70 may drive the increased placental vascular density observed on day 90 in the Meishan uterine environment.

In conclusion, the pig placenta produces significant amounts of VEGF mRNA during late gestation in response to increasing fetal demands. The amount of VEGF is associated with placental efficiency and therefore may be responsible for the degree of placental vascularity. The Meishan pig has the ability to produce conceptuses with small, highly vascular placentae with a resultant increase in placental efficiency and litter size compared with Yorkshire females. The difference may stem from greater stimulation of placental VEGF mRNA and protein potentially through an endometrial-induced hypoxia.